BIODIESEL PRODUCTION BY ENZYMATIC TRANSESTERIFICATION OF OLIVE OIL

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View of Administrative Building

OUTLINE

- Introduction
- Objectives
- Results
  - Parametric studies
  - Feasibility studies
  - CoFoam Comparison
- Conclusions

INTRODUCTION

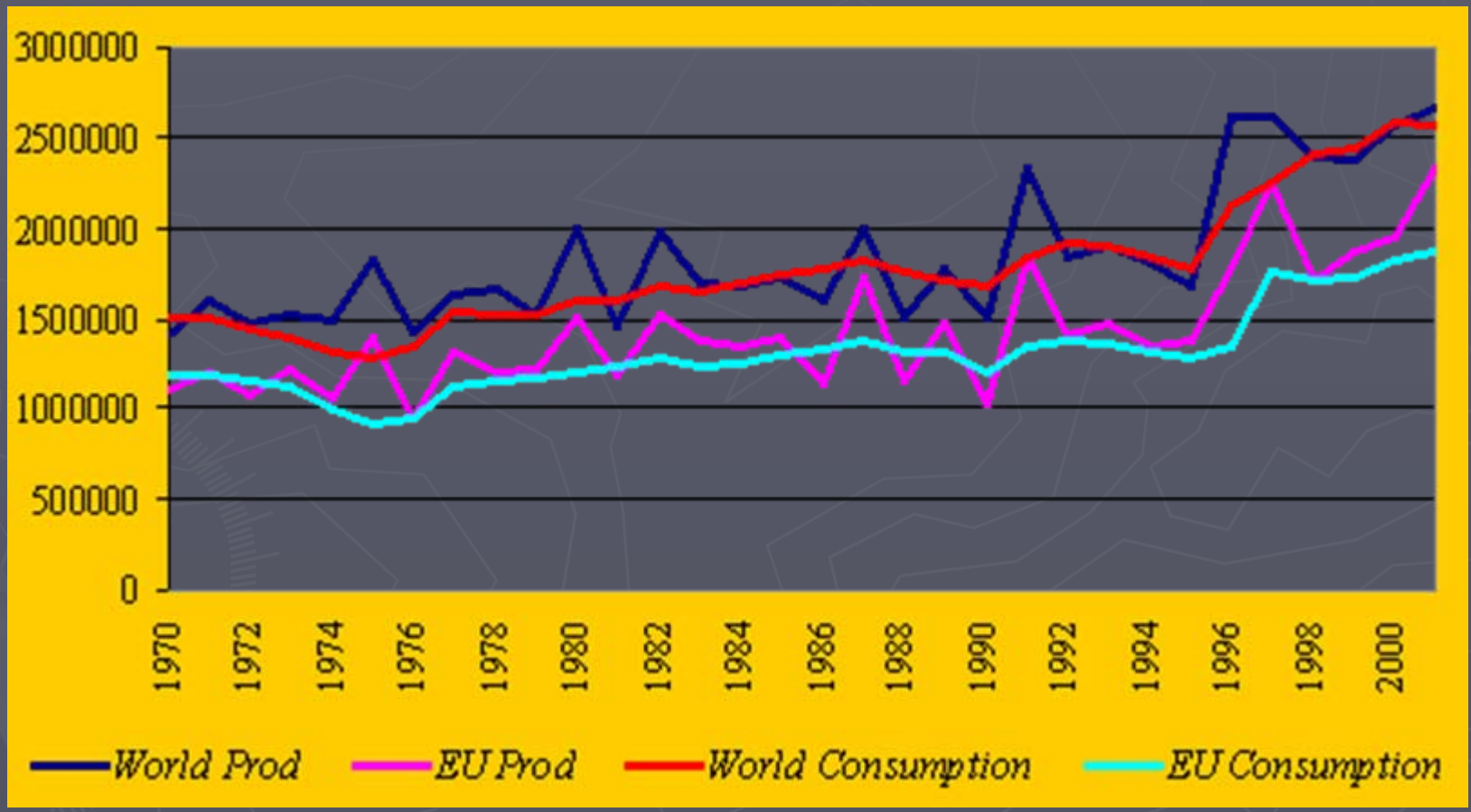
BIODIESEL

- UNH has established a biodiesel group. Focus is on micro algae as this is seen as the best option of producing biodiesel in large quantities
  - Algae can yield 5000-20000 gallons/acre/year
  - Grows best off of waste streams
- Chemical processes for production are well known
- Transesterification of olive oil using lipase

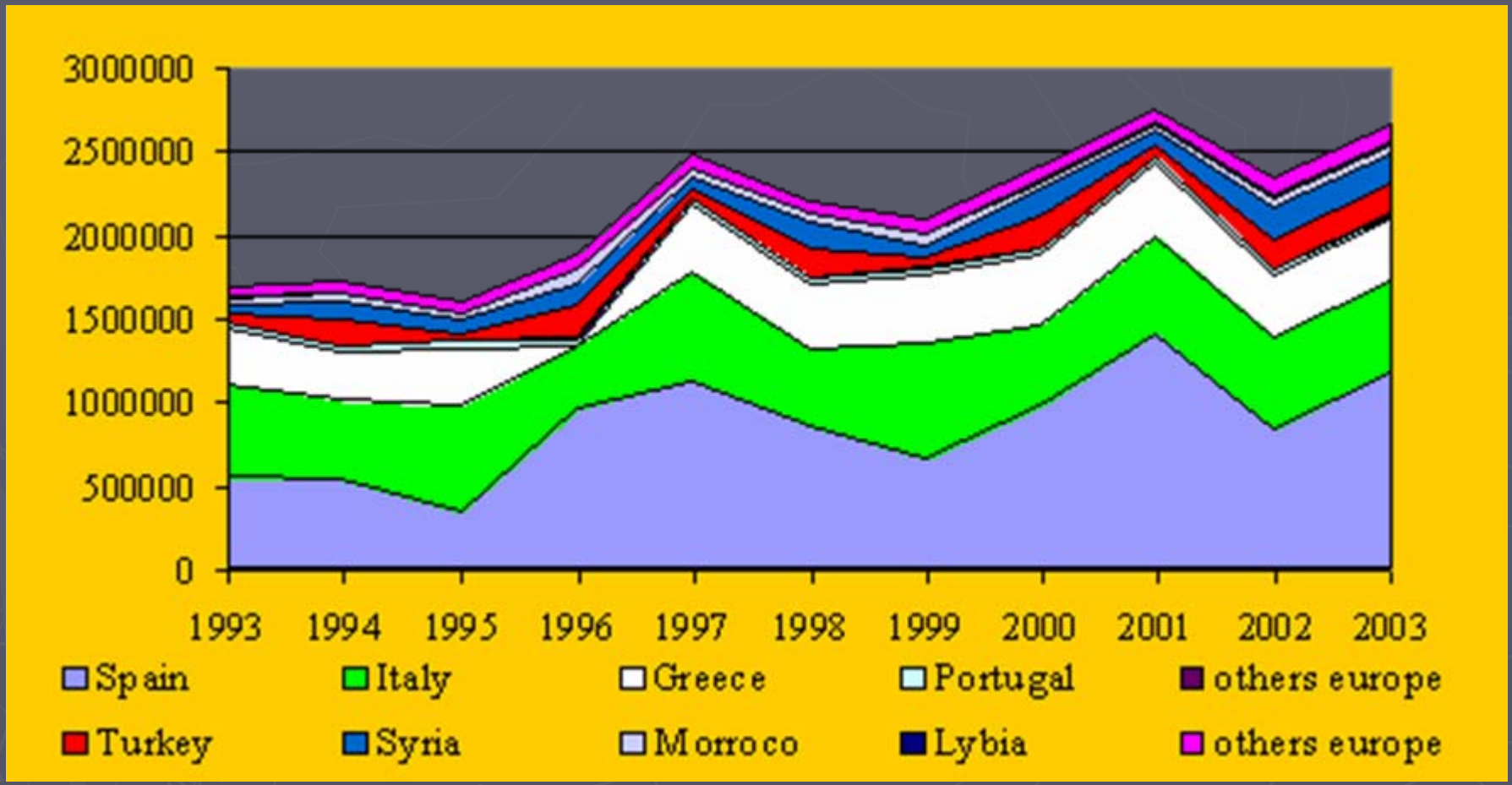
Enzymatic transesterification using different alcohols and lipases

Oil	Alcohol	Lipase	Conv. (%)	Solvent	Ref.
Rapeseed	2-Ethyl-1-hexanol	C. rugosa	97	None	3
Mowrah, Mango, Kernel, Sal	C_4-C_{18} alcohols	M. miehei (Lipozyme IM20)	86.8-99.2	None	3
Sunflower	Ethanol	M. miehei (Lipozyme)	83	None	3
Fish	Ethanol	C. antarctica	100	None	3
Recycled restaurant grease	Ethanol	P. cepacia (Lipase PS-30) + C. Antarctica (Lipase SP435)	85.4	None	3
Tallow, Soybean, Rapeseed	Primary alcohols[a] Secondary alcohols[b] Methanol Ethanol	M. miehei (Lipozyme IM60) C. Antarctica (Lipase SP435) M. miehei (Lipozyme IM60) M. miehei (Lipozyme IM60)	94.8-98.5 61.2-83.8 19.4 65.5	Hexane Hexane None None	3
Sunflower	Methanol Methanol Ethanol	P. fluorescens	3 79 82	None Pet ether None	3
Palm kernel	Methanol Ethanol	P. cepacia (Lipase PS-30)	15 72	None None	3
Canola	Methanol	Novozym 435	97.9	Water	1
Soybean	Methanol Ethanol	P. cepacia Lipase	67 65	Water	5
Castor	Ethanol	Novozym 435 Lipozyme 1M	81.4 <98	Hexane	2

Olive Oil Production and Consumption



Production by Country



INTRODUCTION

TRANSESTERIFICATION REACTION:

Triglyceride Alcohol Fatty acid esters Glycerol

GOALS

- Ensure that enzyme retains activity
- Minimize enzyme denaturation
- Evaluate long-term performance of Novozym®435
- Investigate used-oil utilization
- Scale-up and kinetics

Materials and Methods

- Triglyceride: triolein
- Catalyst: Novozym®435, lipase B from *Candida antarctica* from Sigma Aldrich
- Hexane, methanol, ispropanol – HPLC grade
- Methyl oleate, triolein standards
- Reverse Phase C18 HPLC coupled to UV detector
- UV Absorbance detection at 230 nm

Gradient HPLC Method

Time (min)	Methanol (%)	Isopropanol (%)	Hexane (%)	Flow Rate (m^3/s)
0	100	0	0	2.5e-9
19	100	0	0	2.5e-9
20	100	0	0	8.33e-9
35	64	20	16	8.33e-9
46	64	20	16	8.33e-9

Experimental Set-Up

- Conversion studies were performed in a batch reactor
- Reactor consisted of a glass vial with a poly butyl terephthalate open-top cap and a PTFE-silicone septum.
- The vial measured 0.025 m in diameter by 0.057 m in height.
- Vial placed in a constant-temperature bath and contents were mixed by a stir bar

Reaction Procedure

- Triolein conversion analyzed every 2.5 h for the first 10-12.5 h of reaction.

- Final sample drawn after 20-30 h to check for final conversion.

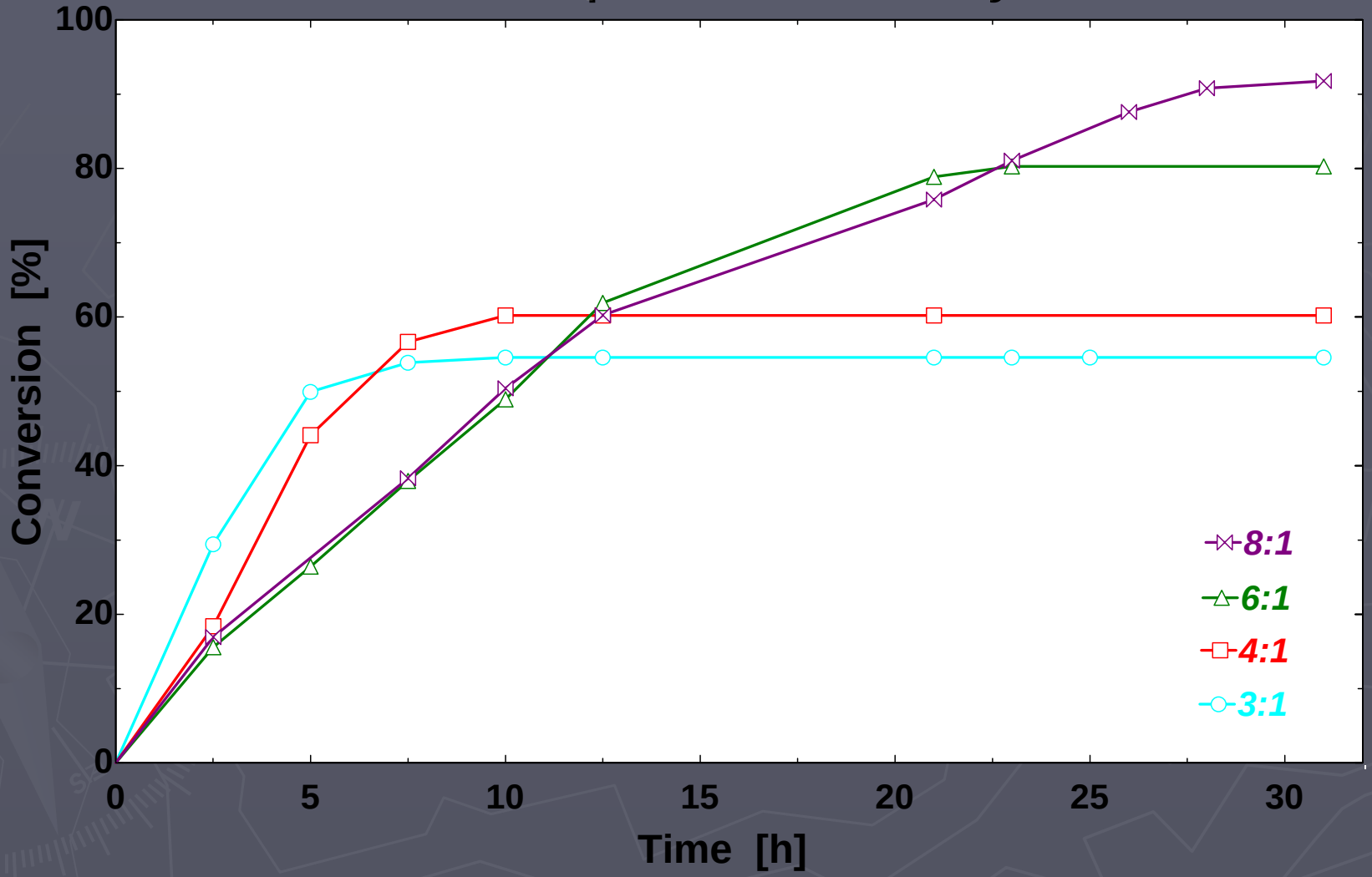
PARAMETRIC STUDIES

- Molar ratio of methanol to triolein
- Semi-batch (step-wise addition) vs batch operation
- Enzyme activity
- Mixing speed
- Reaction temperature

EFFECT OF MOLAR RATIO OF REACTANTS

- Stoichiometry requires 3:1 ratio for triolein to be converted
- Mixtures with more than 3:1 deactivate Novozym®435
- Compromise solution between reactant availability and enzyme denaturation

EFFECT OF MOLAR RATIO ON CONVERSION
40C, 100 rpm, 500 U enzyme

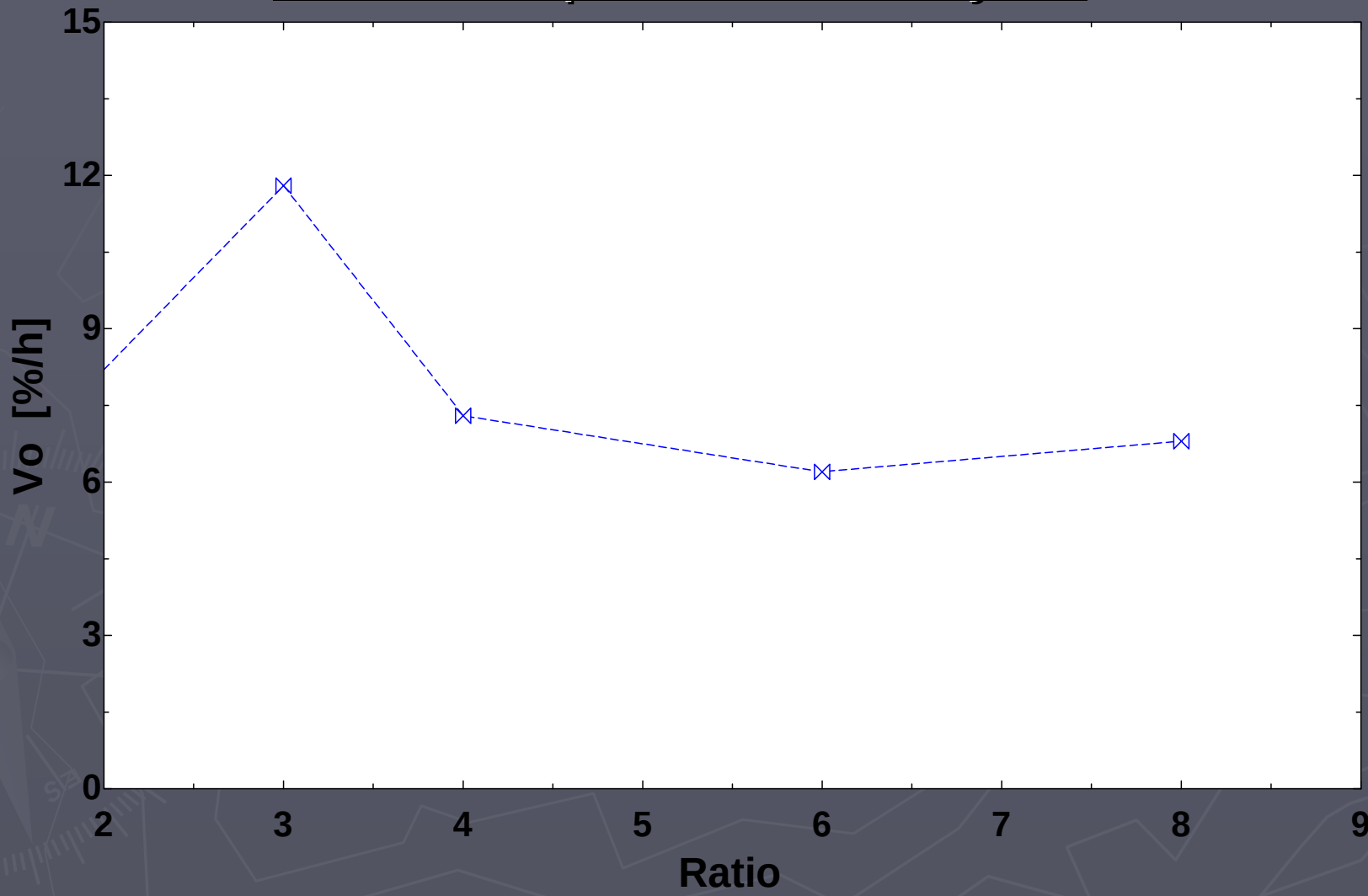


Observation and Inference

- The initial rates (slopes of the curves during the first 2.5 h) at ratios 4:1, 6:1 and 8:1 are almost identical, but lower than the rate corresponding to a ratio of 3:1.
  - This indicates that the extent of inhibition of Novozym®435 by methanol is limited and remains constant for methanol to triolein ratios greater than 4:1.

- Methanol in excess of the stoichiometric amounts leads to higher final conversions of triolein.
  - Attributed to the presence of other components in olive oil that also compete for methanol. Presence of other products (not identified) was revealed during analysis. Thus when methanol is present in excess, there is enough reactant available for almost complete conversion of triolein.

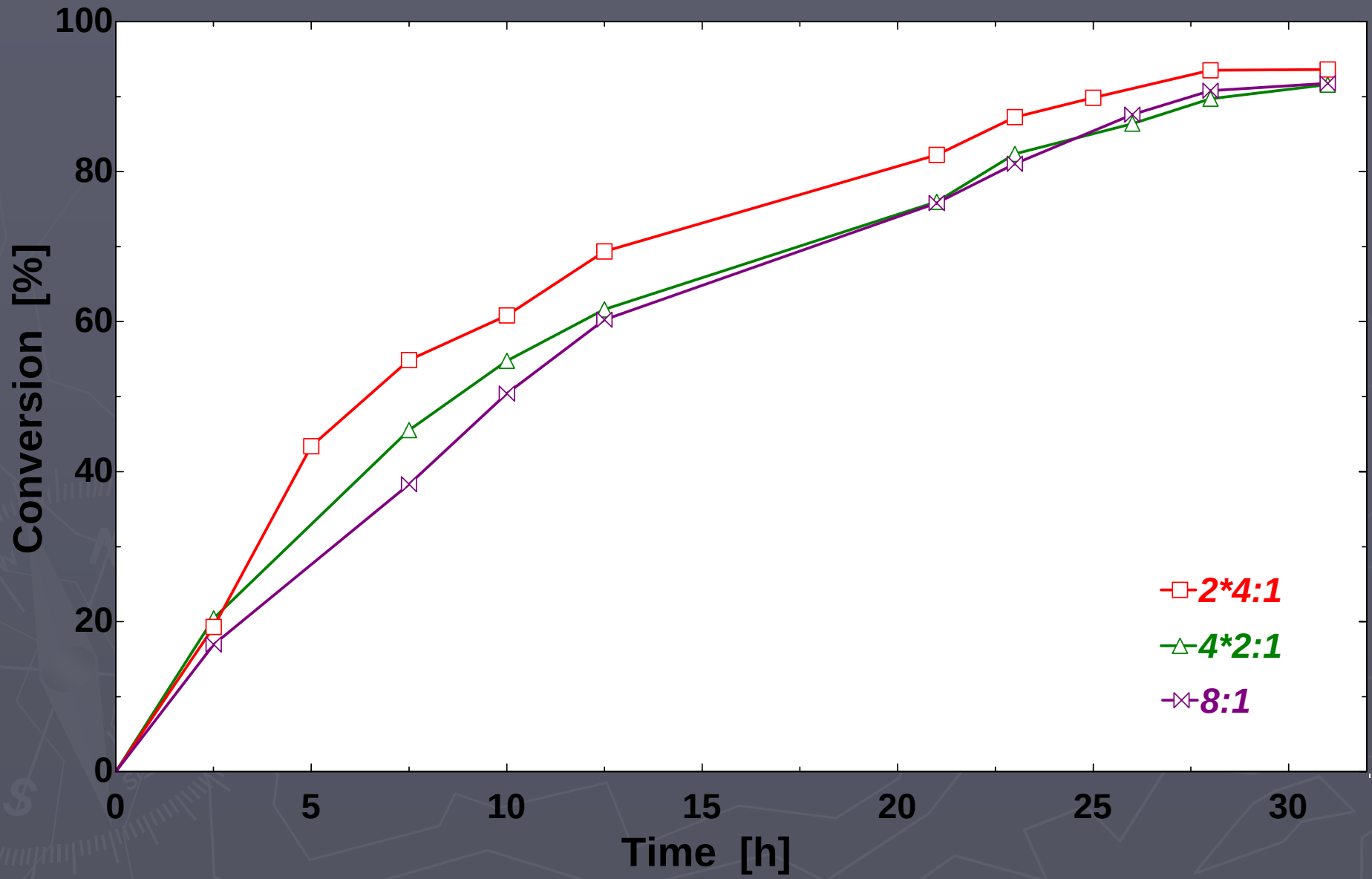
EFFECT OF MOLAR RATIO ON RATE
40C, 100 rpm, 500 U enzyme



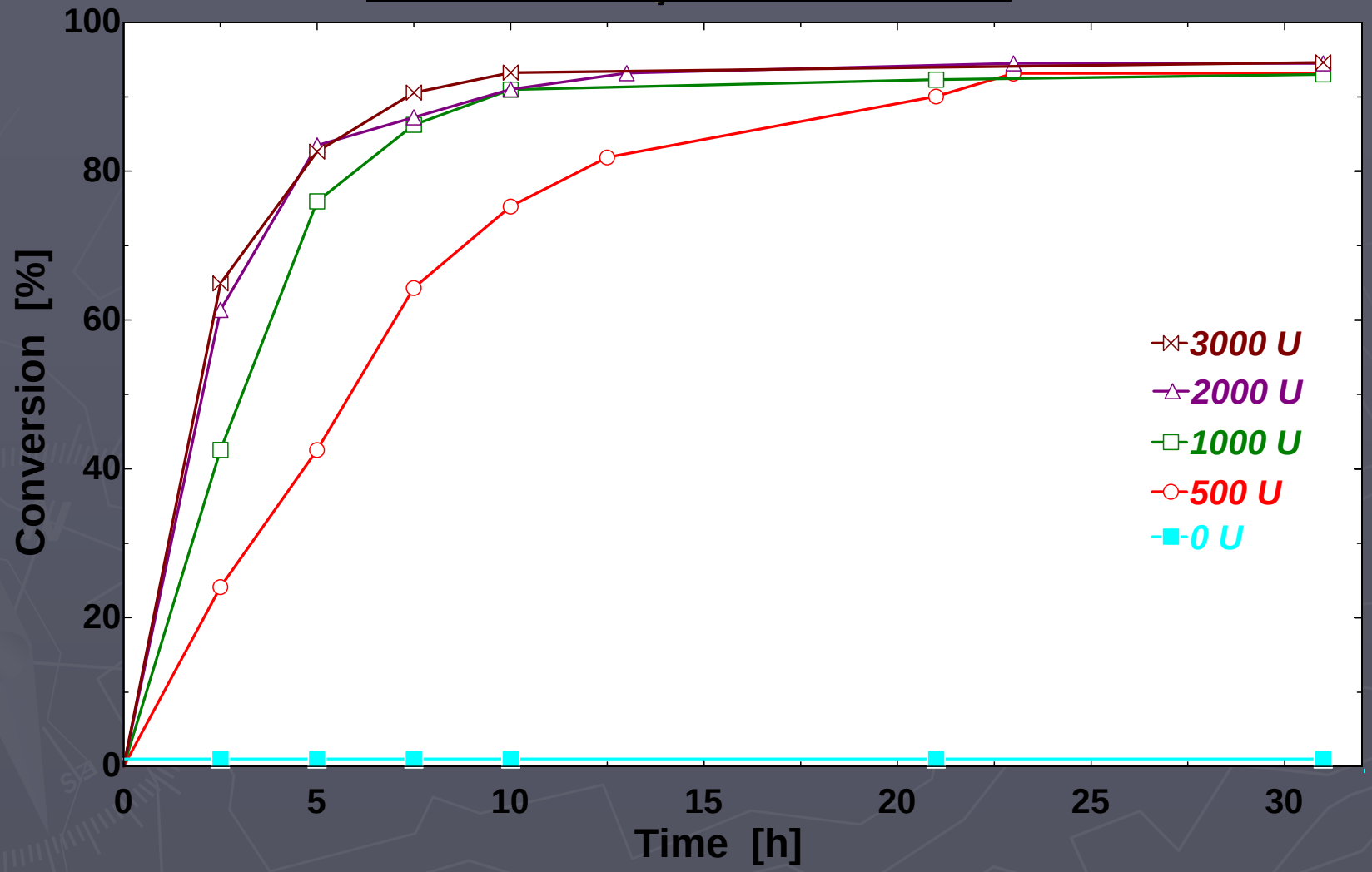
Effect of methanol to triolein molar ratio

Ratio	Initial rate x 10^3 (mg/U-s)	Final conversion (%)	Yield (g biodiesel/g oil)
8:1	1.4	91.8	0.41
6:1	1.3	80.3	0.36
4:1	1.5	60.2	0.27
3:1	2.4	54.6	0.25

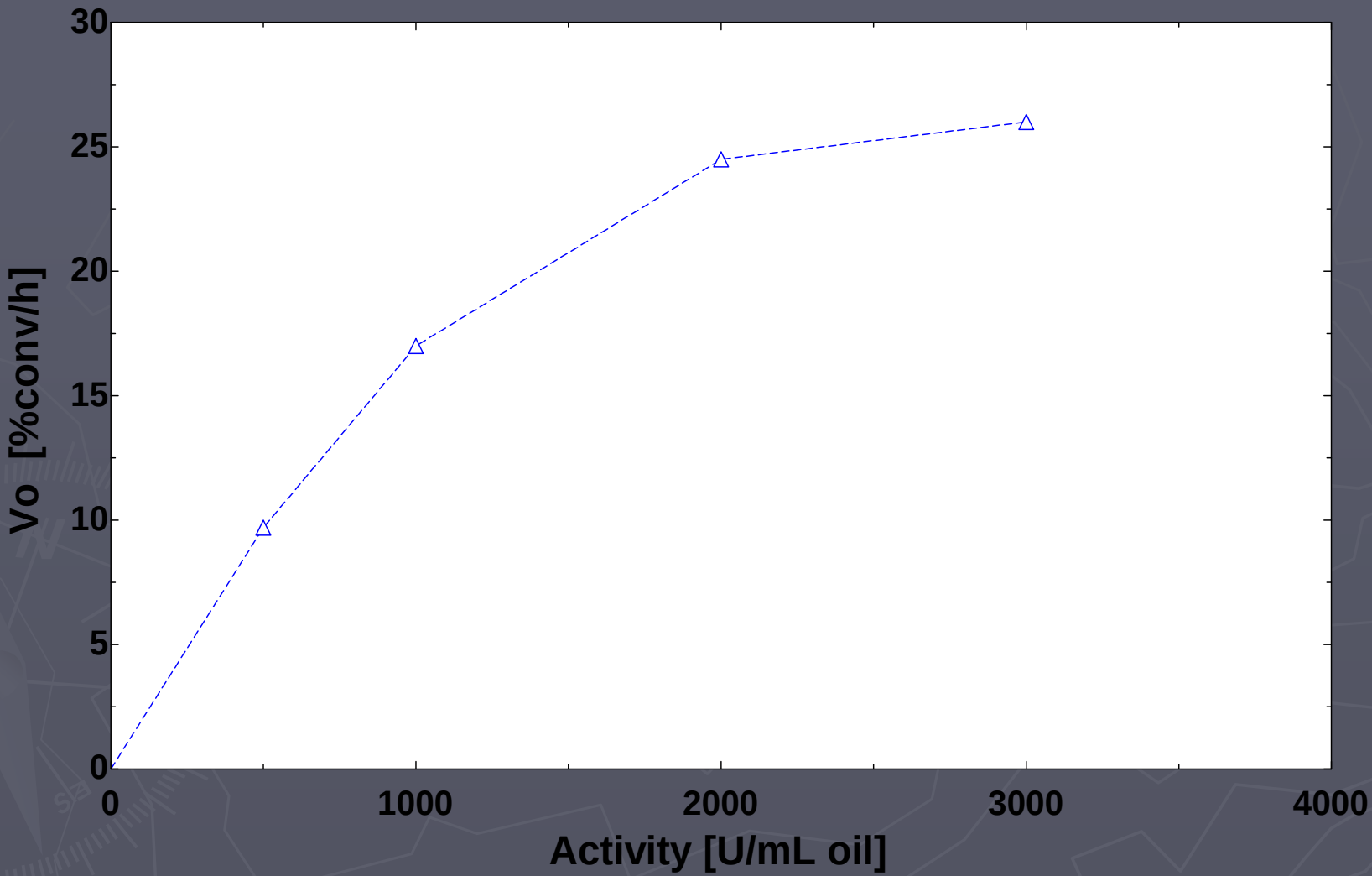
SEMI-BATCH VS BATCH – EFFECT ON CONVERSION



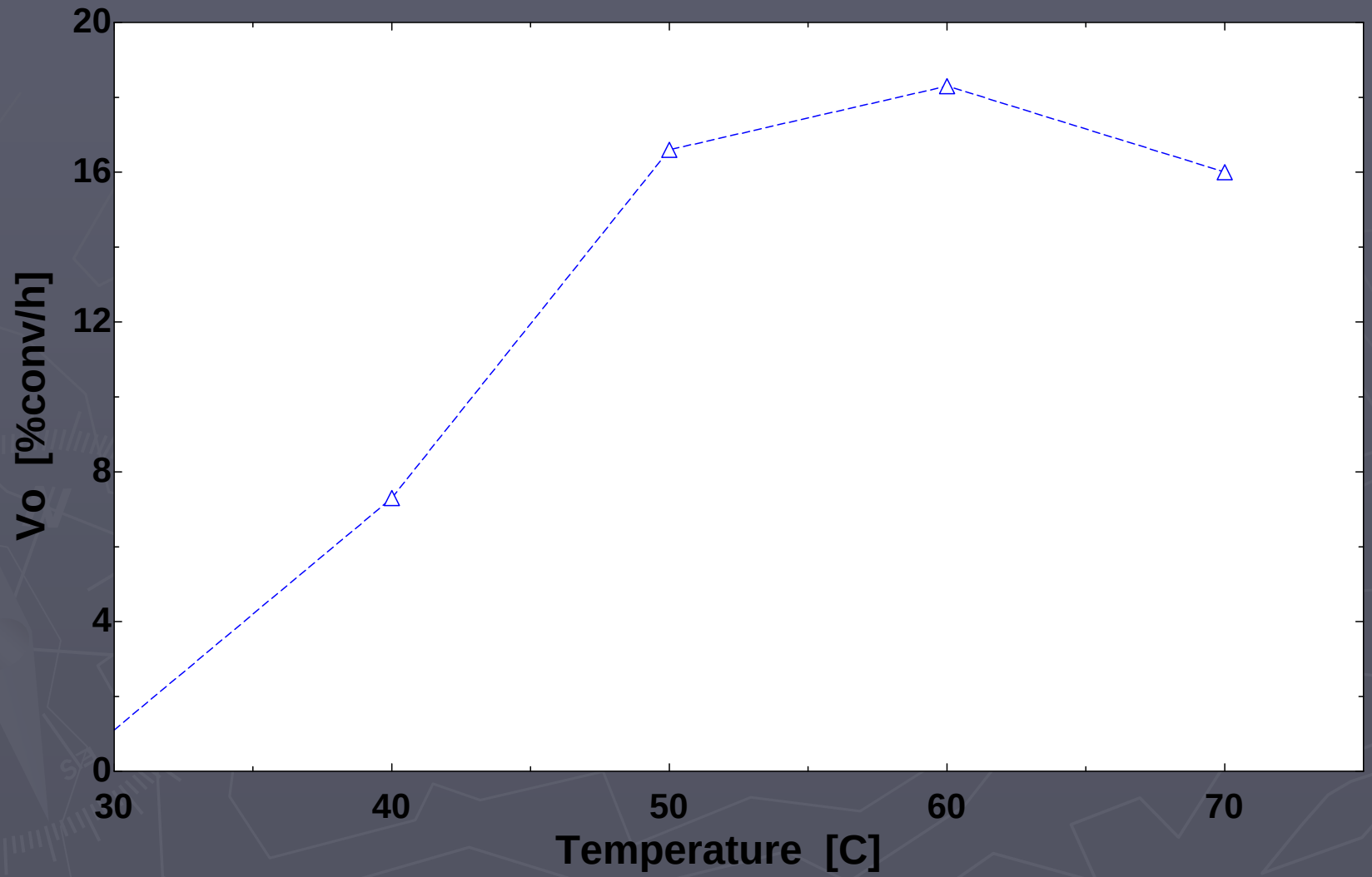
EFFECT OF ENZYME ACTIVITY ON CONVERSION
60C, 100 rpm, 8:1 ratio



EFFECT OF ENZYME ACTIVITY ON RATE



EFFECT OF TEMPERATURE ON RATE
500 U enzyme, 100 rpm, 4:1 ratio



Effect of Temperature

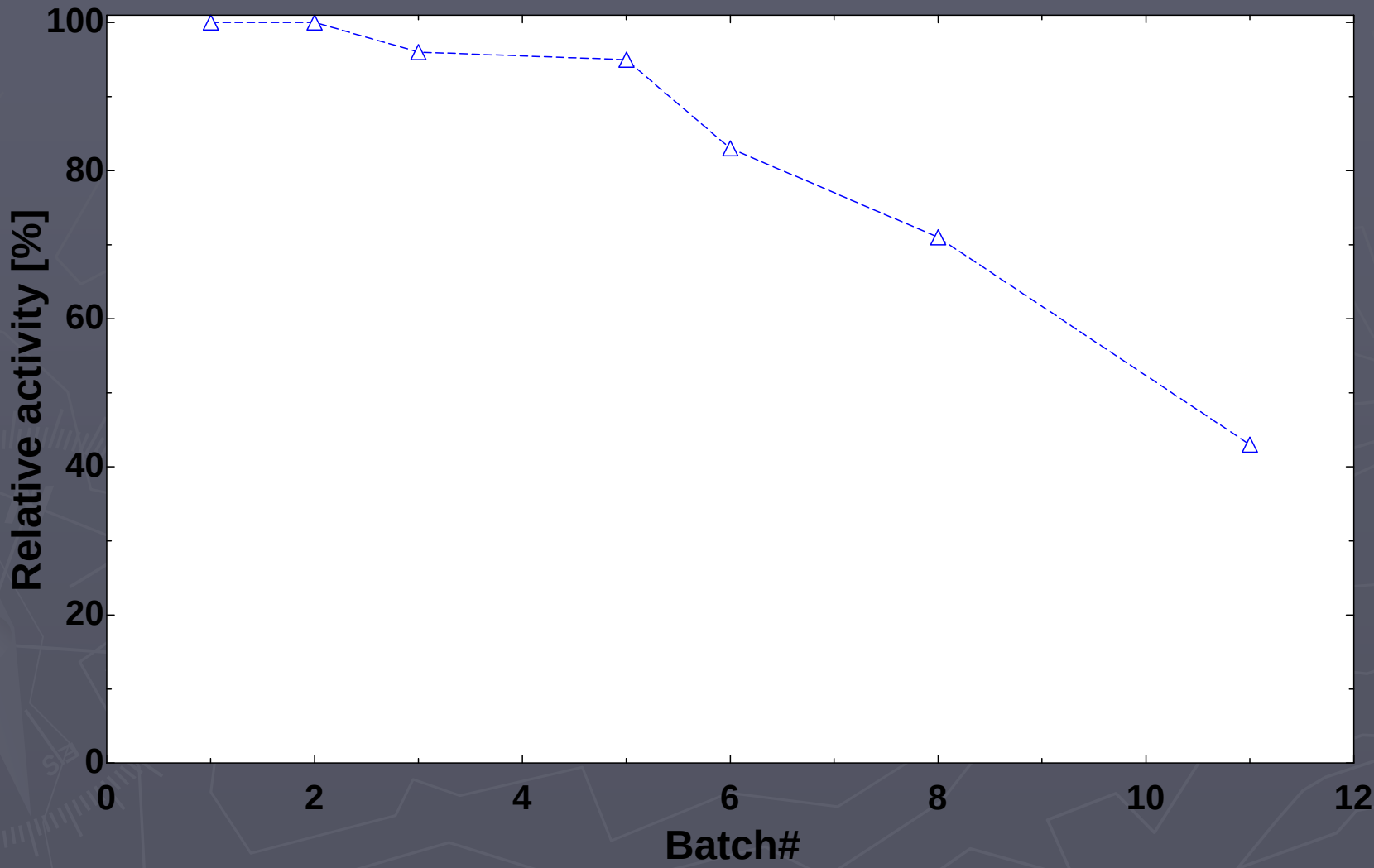
Temperature (ºC)	Initial rate x 10^3 (mg/U-s)	Final conversion (%)	Yield (g biodiesel/g oil)
30	0.23	31.7*	Not calculated
40	1.53	60.2	0.27
50	3.44	58.4	0.26
60	3.81	59.3	0.27
70	3.33	53.4	0.24

FEASIBILITY: REUTILIZATION OF ENZYME

- Major drawback compared to chemical catalysts: Cost
- This reaction: raw materials and energy costs are low

  ⇒ enzymatic catalyst: main expense
- Glycerol has negative effects on lipase activity

FEASIBILITY: REUTILIZATION OF ENZYME
60C, 1000 U enzyme, 8:1 ratio



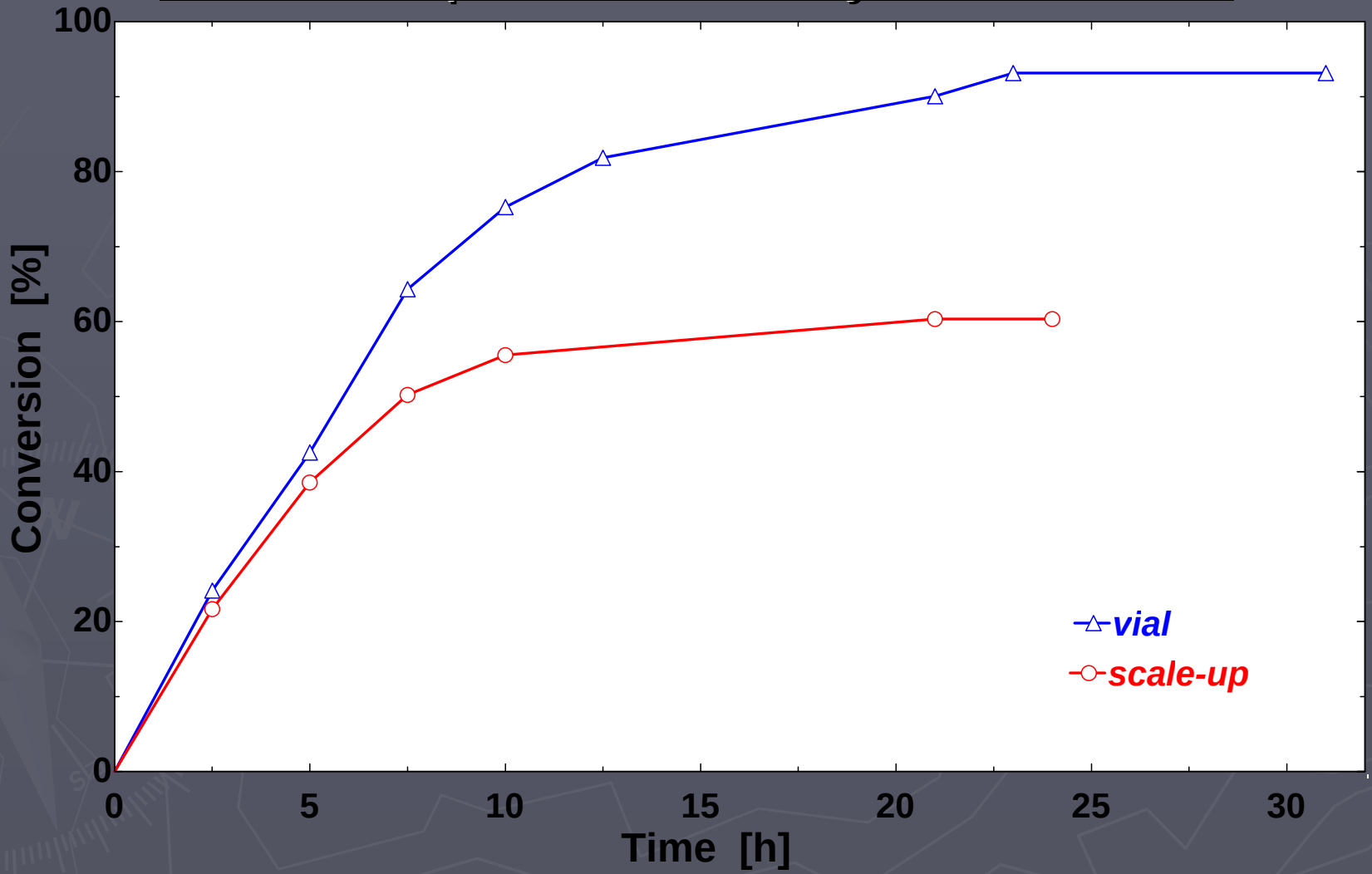
Effect of Enzyme Reutilization

Batch	Relative Activity (%)	Yield (g biodiesel/g oil)	Productivity·10^5 (g biodiesel/U enzyme-h)
1	100	0.42	3.96
2	100	0.41	3.96
3	96	0.40	3.69
5	95	0.42	3.69
6	83	0.41	3.62
8	71	0.41	3.42
11	43	0.34	2.40

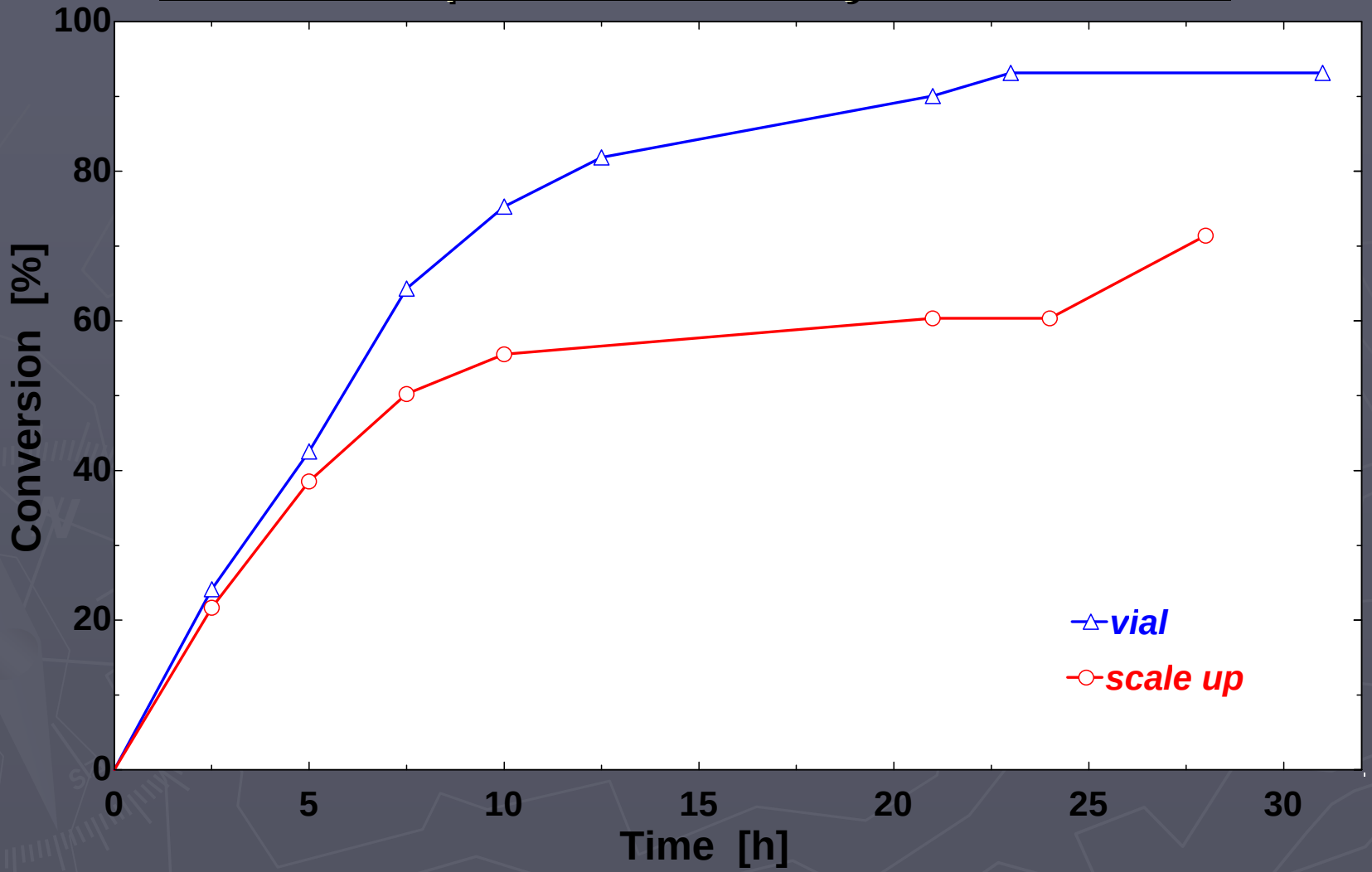
FEASIBILITY: SCALE-UP

- Next objective: to assess biodiesel production in larger reactor
- Physical conditions in large reactor rarely duplicate those in smaller one → heat transfer, diffusion… → overall reaction rate
- Change in controlling regime → results from small scale become unreliable to predict those in large scale

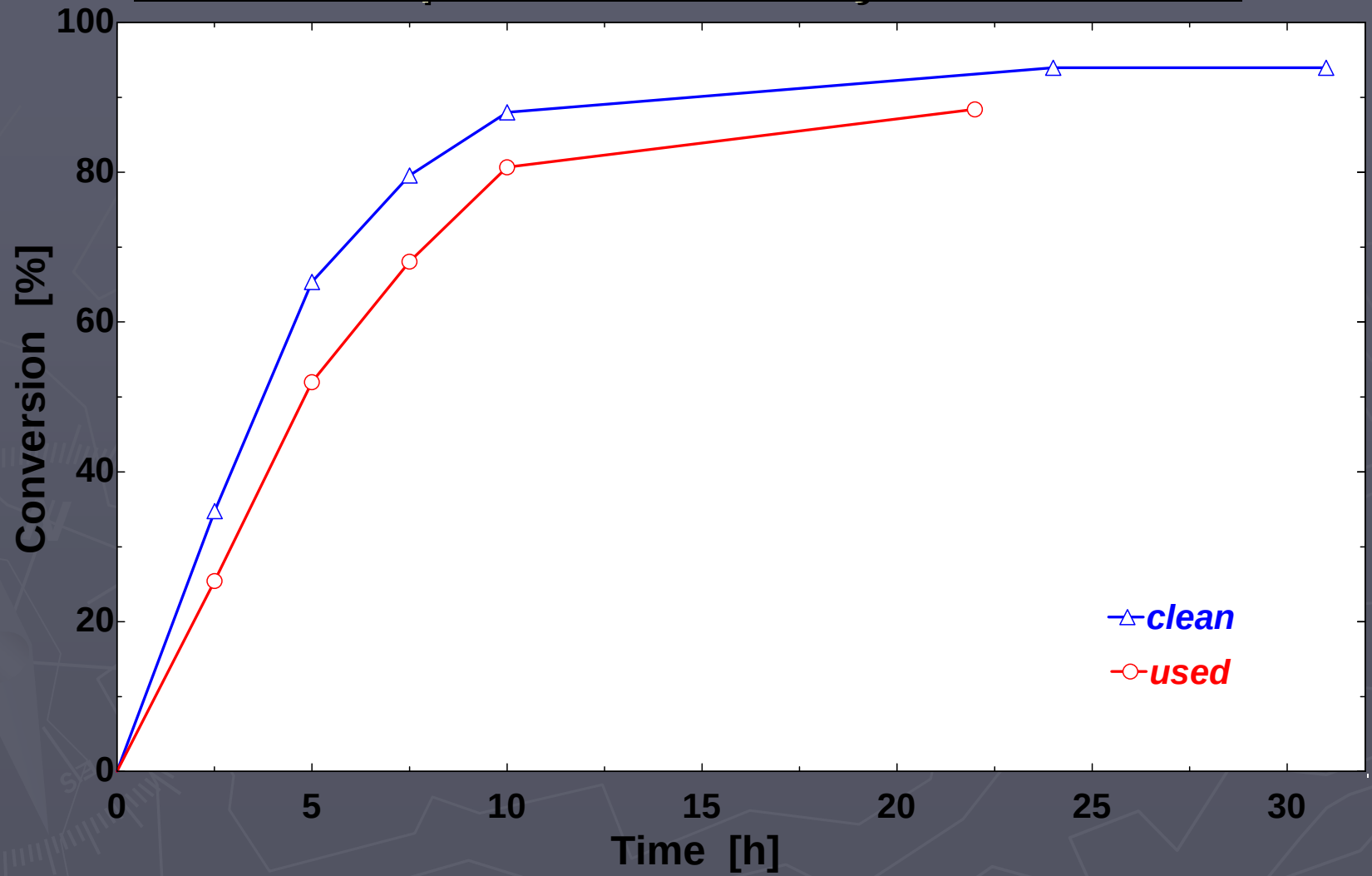
SCALE-UP: Conversion versus Time
60C, 100 rpm, 500 U enzyme, 8:1 ratio



SCALE-UP: Effect of Methanol Addition 60C, 100 rpm, 500 U enzyme, 8:1 ratio



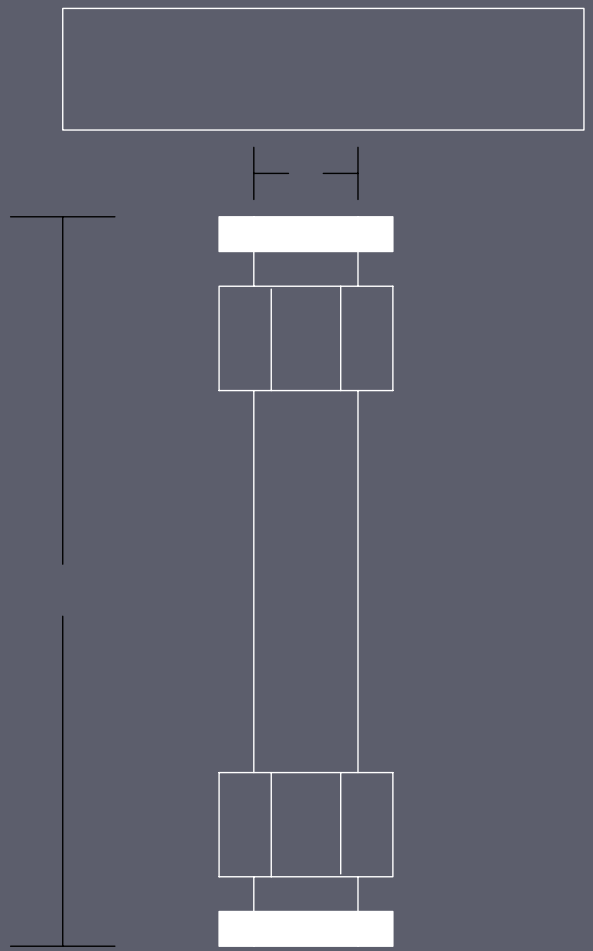
FEASIBILITY: USED OIL
60C, 100 rpm, 1000 U enzyme, 8:1 ratio



Work in Progress

- New reactor design that has virtually eliminated loss of solvent
- Effect of other solvents on yield and conversion
- Effect of ultrasonic agitation on yield and conversion
- New analytical method has been developed that is reliable and cheaper than HPLC

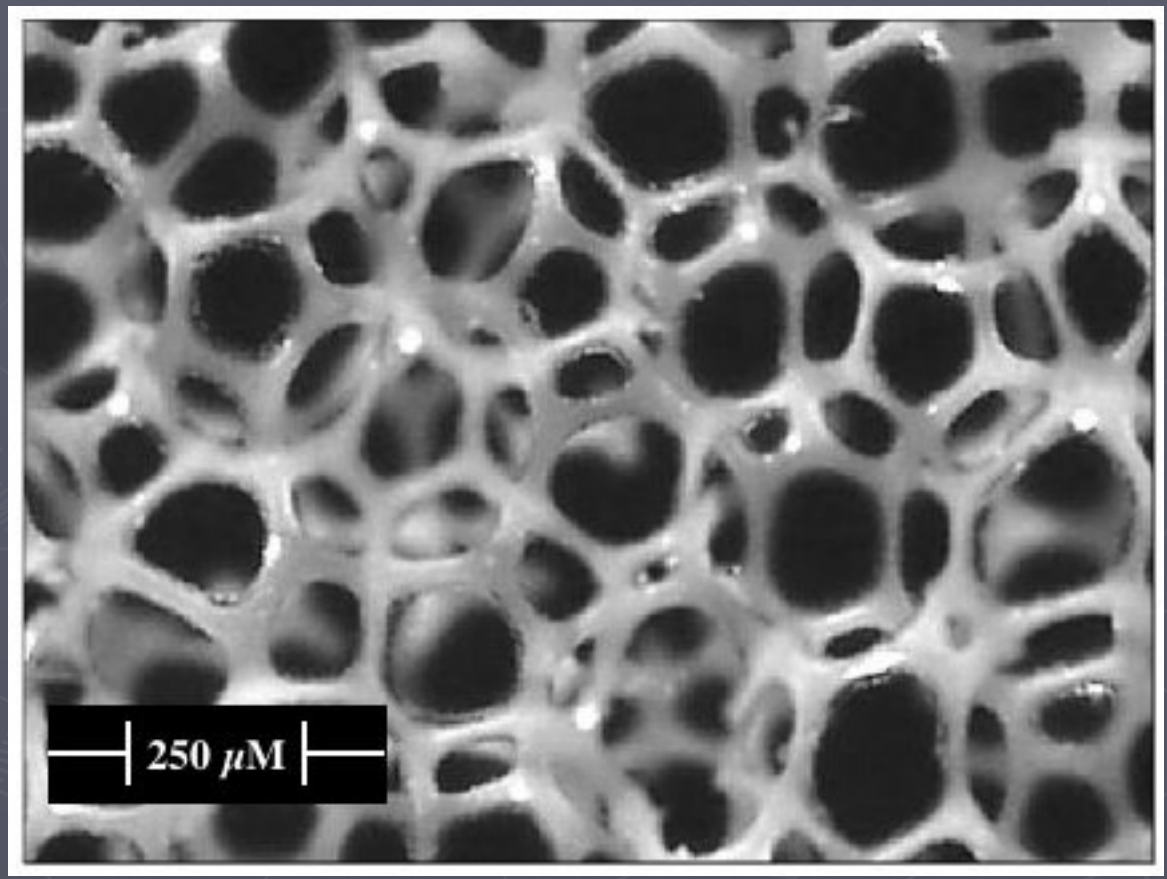
New Reactor



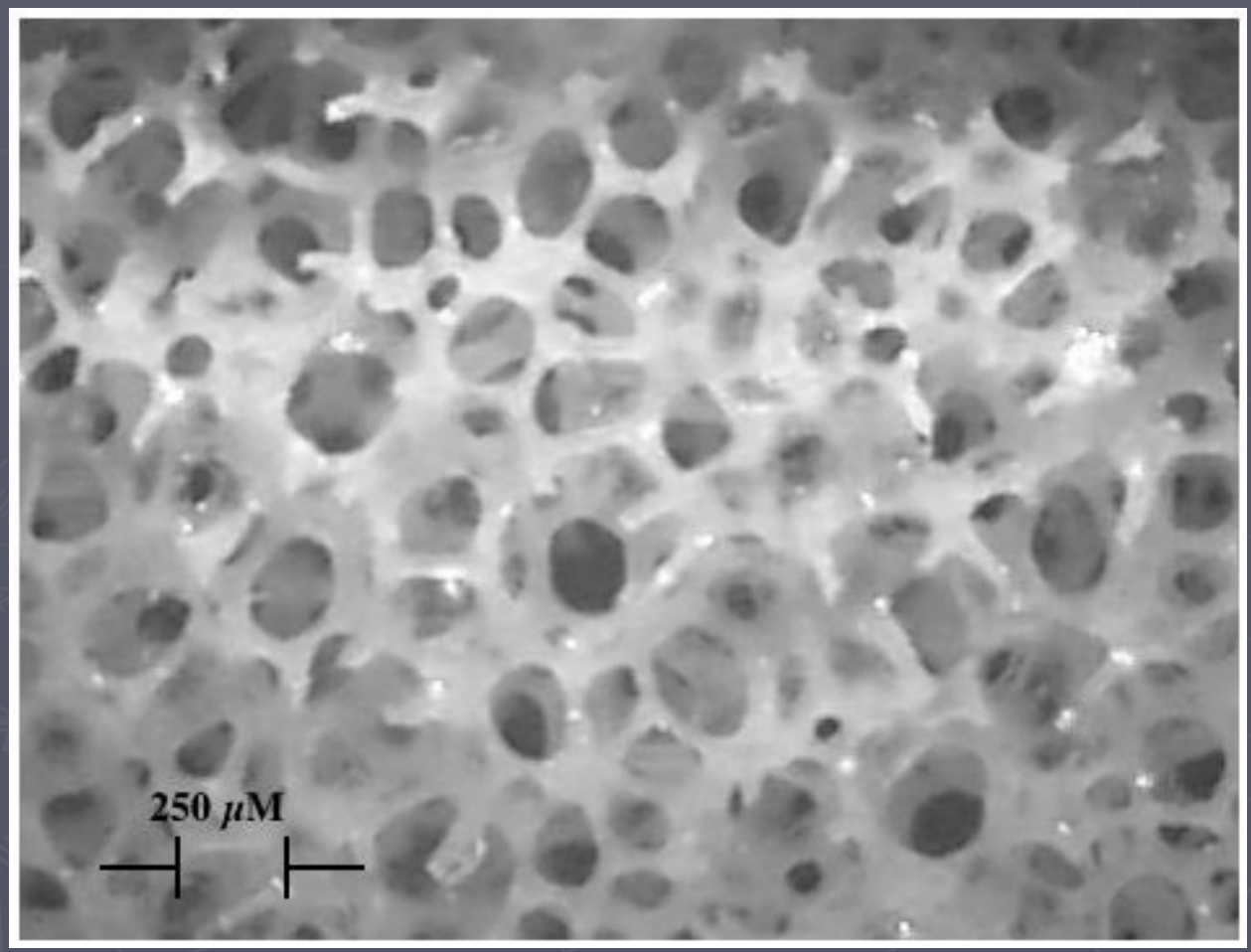
Other Supports

- Developed an effective technique to immobilize enzymes on hydrophilic CoFoam.
- Hydrophilic polyurethane foam is cast on to reticulated foam
- Works in the temperature range 4C – 107C
- Hydrophilicity, pore size and foam density can be altered
- Enzymes are covalently bound
- Adds strength and there is no need to cut to small pieces to increase surface area

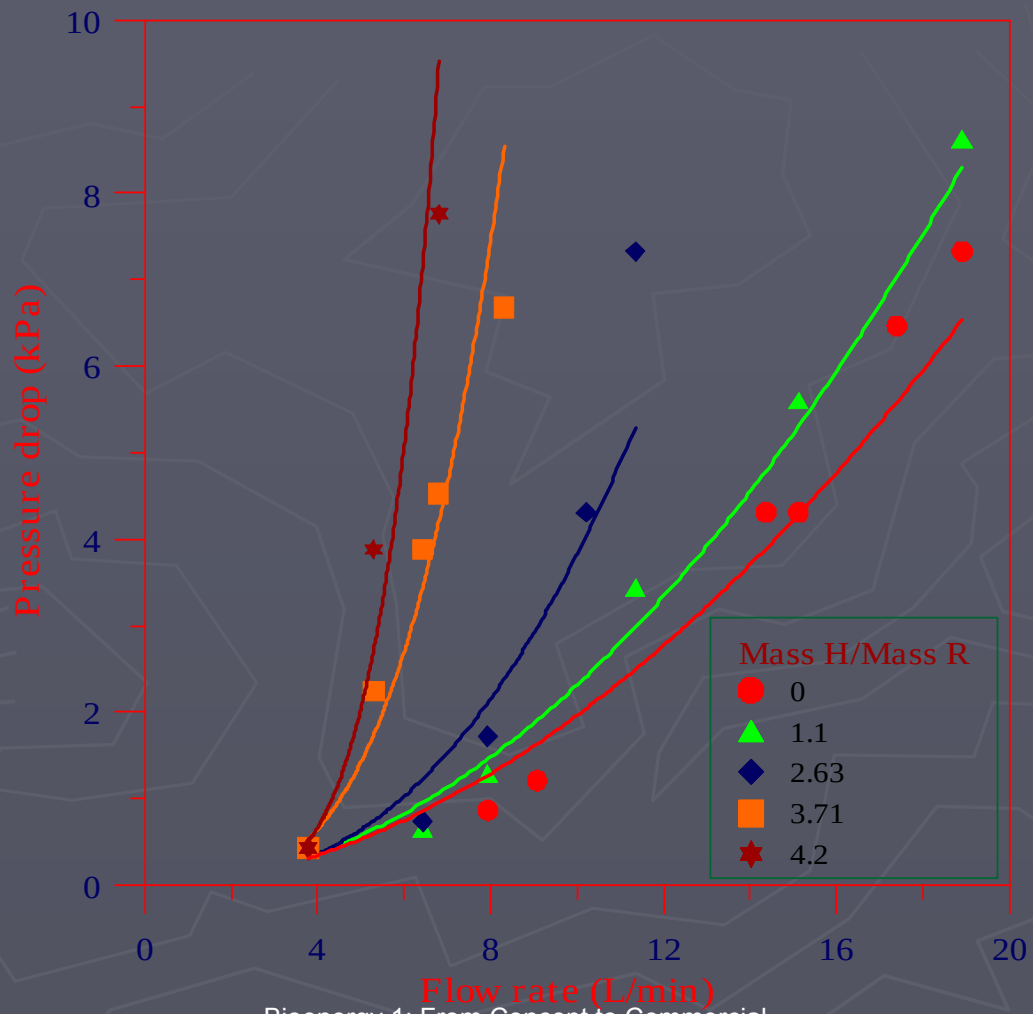
Reticulated Foam



Coated Reticulated Foam



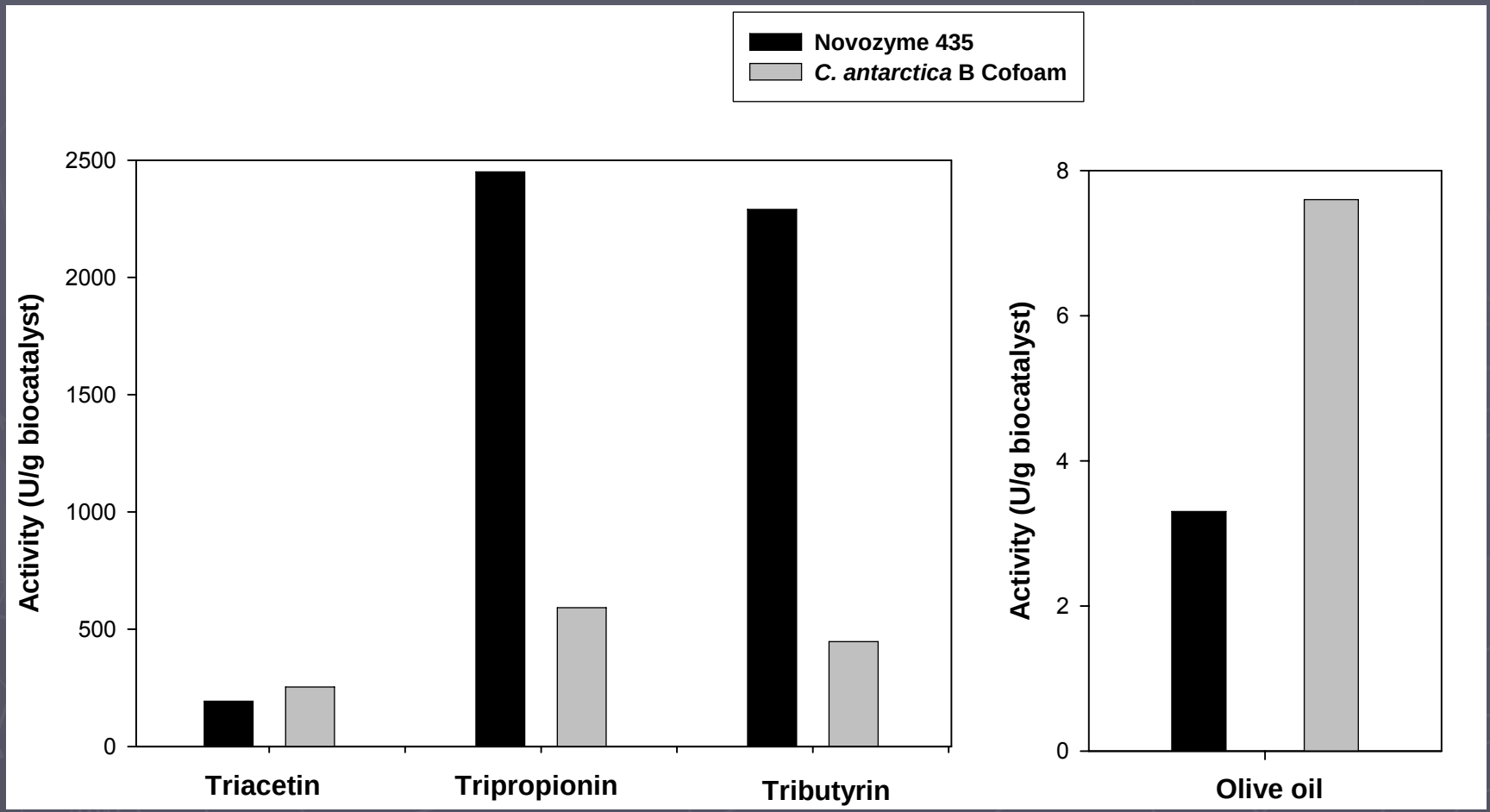
Pressure drop (kPa) vs Flow rate (L/min)



Immobilization of Lipase

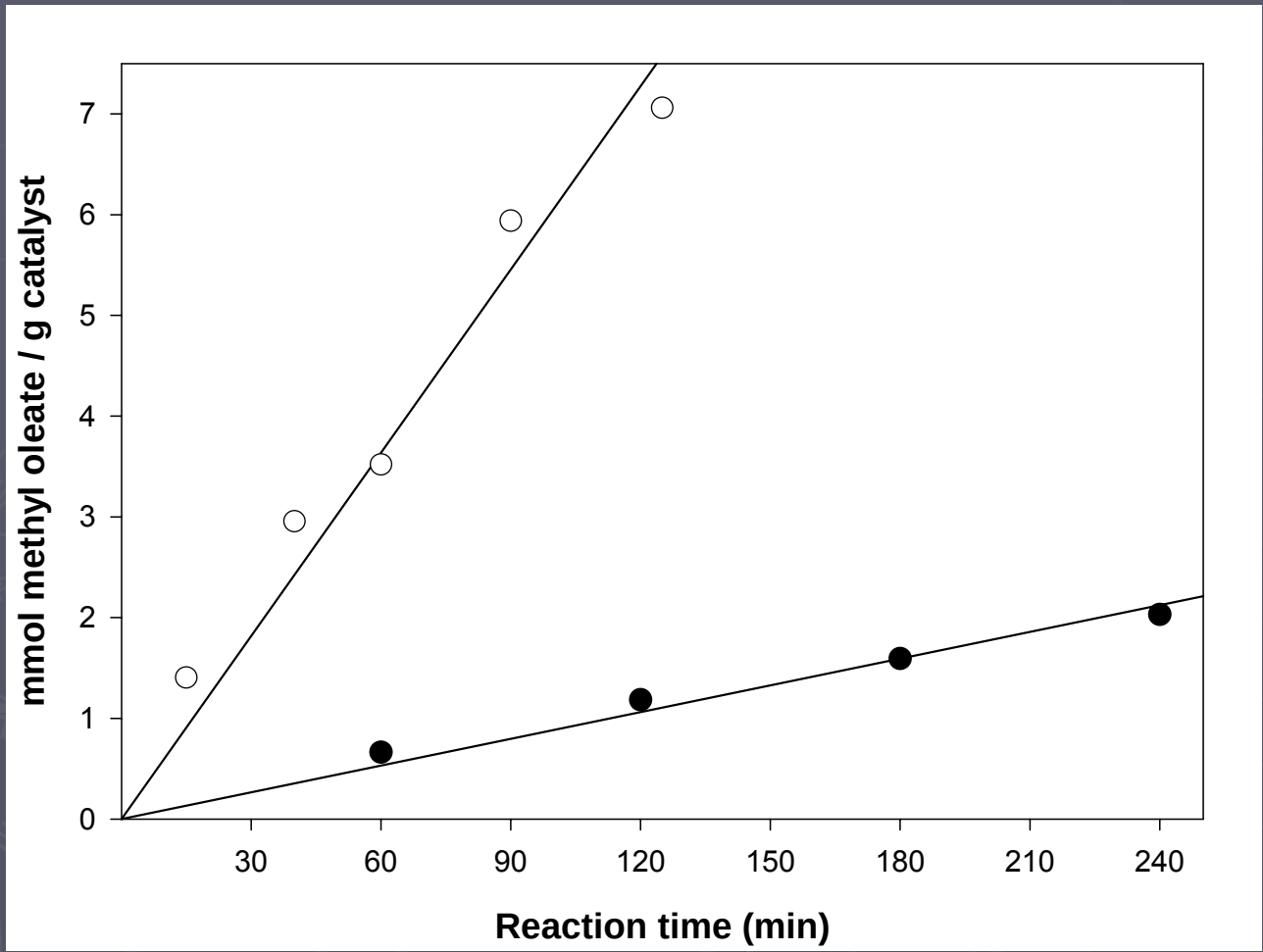
- Video clip

Hydrolytic Activity



Synthetic Activity

● CoFoam ○ Novozyme 435



CONCLUSIONS

- Inhibition of Novozym®435 by methanol is reversible and remains constant for molar ratios greater than 4:1
- Methanol in excess of stoichiometric amounts leads to higher final conversions
- Step-wise methanolysis indicates some improvement
- At enzyme concentrations higher than 2000 U/mL oil, addition of catalyst barely affects reaction rates
- Mixing speed does NOT play a role within 50-400 rpm

CONCLUSIONS

- Initial reaction rate reaches a maximum at approx. 60C
- 95% activity retained after 5 batches, >70% after 8
- Under similar conditions, results can be extrapolated to large scale and used oil within a margin of 10-15%
- First order kinetics with respect to oil concentration

Acknowledgements

- Hydrophillix, Inc.
- Dr. Francisco Plou, ICP for hydrolytic activity measurements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REG UNLD
BioDiesel
ALL TAXES INCLUDED
WELCOME
NEWMARKET
Optina
Day Spa

QUESTIONS